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IN THE UNITED STATES PATENT AND TRADEMARKS OFFICE

In The Matter Of Patent Application:

Applicant	:	Michael J. ROCHON
Assignee	:	Virox Technologies Inc.
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Title	:	HYDROGEN PEROXIDE DISINFECTANT WITH INCREASED ACTIVITY
Examiner	:	John Pak
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To: Assistant Commissioner for Patents
The U.S. Department of Commerce,
PATENT OFFICE
Washington, D.C., U.S.A., 20231

AFFIDAVIT OF JOSE A. RAMIREZ

I, Jose A. Ramirez, of the City of Mississauga, in the Regional Municipality of Peel, MAKE OATH AND SAY:

1. I was the vice President of Research and Development of the assignee of the present invention, Virox Technologies Inc. I have M.S. and Ph.D. degrees in Chemical Engineering and 12 years of experience working in the field of chemistry and chemical engineering. Furthermore, I am a Member of the American Institute of Chemical Engineers and the Canadian Institute of Chemistry. As such, I have personal knowledge of the following matters to which I depose.

2. I have conducted experiments to study the contribution of individual components of the present inventive formulation towards overall efficacy. The following legend applies to assist in the understanding of Examples A to E below.

Phosphorous-based compounds

- **H_3PO_4** = phosphoric acid
- **STPP** = sodium tripolyphosphate
- **BRIQUEST ADPA-60AW (HEDP)** = 1-hydroxyethylidene-1,1,-diphosphonic acid
- **Briquest 301-50A (ATMP)** = amino tri(methylene phosphonic acid)

Anionic surfactants

- **Biosoft S-100 (DDBSA)** = dodecyl benzene sulfonic acid
- **Dowfax C10L C10** = C10 alkylated sulfonated diphenyl oxide disodium salt
- **Petro ULF (ANS)** = sodium alkyl naphthalene sulfonate
- **Bioterge PAS-8 (SOS)** = sodium octyl sulfonate
- **Hostapur SAS-30** = sodium C14 - C17 sec-alkylsulfonate
- **Stepanol WAC (SLS)** = sodium lauryl sulfate
- **Standapol LF (SOS)** = sodium octyl sulfate
- **Stepan Mild SL3 (SLSS)** = disodium laureth sulfosuccinate
- **DOWFAX hydrotrope** = C6 alkylated sulfonated diphenyl oxide disodium salt
- **Alpha-Step MC-48 (SMSE/SFA)** = solution containing SMSE and SFA (relative ratio of components not given by manufacturer)
  - SMSE = sodium methyl 2-sulfo C_{12}-C_{16} ester
  - SFA = disodium 2-sulfo C_{12}-C_{18} fatty acid salt

Non-ionic surfactants (emulsifiers)

- **Alfonic L610-3.5** = C6 - C10 alkyl, 3.5 moles of ethylene oxide (EO) alcohol ethoxylate (AE)
- **TRITON X-405 (OPE)** = octyl phenol ethoxylate

Anionic surfactants (hydrotropes)

- **C6 DOWFAX hydrotrope** = C6 alkylated sulfonated diphenyl oxide disodium salt

EXAMPLE A

3. Formulations A1 to A5 were prepared and tested on the gram positive surrogate *Staphylococcus aureus* according to two methods discussed below. The results were compiled and are shown in Table A below.

TABLE A

FORMULATION	A1	A2	A3	A4	A5
INGREDIENT – [%] w/w	% w/w	% w/w	% w/w	% w/w	% w/w
H_3PO_4 (75%)	0.11	0.11	0.11	0.20	0.11
BRIQUEST ADPA-60AW (60% HEDP)	0.28	0.28	0.28	-----	0.29
DOWFAX Hydrotrope (45%)	0.08	0.08	0.08	------	0.08
BIOSOFT S-100 (98% DDBSA)	0.18	0.18	-------	0.18	0.18
TRITON X-405 (70% OPE)	0.06	0.06	0.06	------	0.04
H_2O_2 (50%)	1	------	1	1	0.55
pH	1.91	1.93	1.94	1.97	1.91
Germicidal Results					
AOAC 960.09 suspension test, contact time 30 seconds	6.22	6.04	NM	6.22	>6
Quantitative carrier test of Sattar et al. (1998), contact time 3 min.	6.22	3.43	1.66	6.22	>6

Table A. Single-factor experiments with 1% and 0.55% hydrogen peroxide. Numbers shown under germicidal results are Log_{10} reduction in the number of viable organisms. NM: not measurable due to substantial growth - plates had colonies too numerous to count (typically a reduction of less than $3Log_{10}$). A 6-Log_{10} reduction is considered effective as a disinfectant.

4. In these experiments, two methods of testing germicidal activity identified above were used. The AOAC 960.09 method is a suspension test standardized by the AOAC, Association of Official Analytical Chemists, which uses a contact time of 30 seconds. This method gives rise to more favourable disinfection results than the second method and involves adding a small drop of the organism inoculum to a tube of germicide solution and mixing for the specified contact time. The microbes are suspended in and surrounded entirely by the germicide solution. The second method is the carrier test described in Sattar S.A., Springthorpe, S. and M. Rochon, "A product based on accelerated and stabilized hydrogen peroxide: evidence for broad-spectrum germicidal activity", Can. J. Infection Control, Winter 1998. This test is more challenging, giving rise to less favourable

disinfection results than the AOAC 960.09 test. This second method is used in higher risk applications where the presence of even low levels of bacteria cannot be tolerated, such as in the disinfection of surgical instruments used in the health industry. The carrier test involves drying the microbe inoculum on the surface of a carrier (a small glass or steel disc or plate) and then immersing the carrier in the germicide solution. The germicide solution must penetrate a large lumped mass of organism/culture broth in order to effectively reach all microbes and effect a good kill.

5. Although Formulation A4 contains 0.27 wt./wt/ % phosphoric acid as compared to 0.15 wt./wt/ % of Formulations A1 to A3, and A5, I have found in separate experiments (not presently shown) that, at these concentration levels, phosphoric acid does not contribute to the bactericidal activity of the solution. In the above experiments, the phosphoric acid is used to buffer the solution to pH levels slightly below 2 as shown.

6. Formulations A1, A4 and A5 are exemplary embodiments of the present invention containing 1.0, 1.0 and 0.55 wt./wt/ % hydrogen peroxide, respectively. These solutions result in a greater than 6 Log_{10} reduction in bacteria counts, showing them to be effective disinfectants using both methods of testing.

7. Formulation A2 is similar to formulation A1 except that the hydrogen peroxide has been omitted. Using the AOAC 960.09 suspension test, we see that the formulation exhibits a high level of germicidal activity which is attributed to the anionic surfactant, dodecyl benzene sulfonic acid (DDBSA). This is quite expected as concentrations of DDBSA as low as 200 ppm are known to be effective in sanitizing (cf. Lopes, J.A. (1986) "Evaluation of diary and food plant sanitizers against Salmonella typhimurium and Listeria monocytogenes", J. Dairy Sci., 69, 2791-2796). However, when the more challenging quantitative carrier test of Sattar et al. (1998) is used, it is apparent that both the DDBSA and the hydrogen peroxide are necessary to achieve disinfection.

8. The results for formulation A3 show that the anionic surfactant, DDBSA, is necessary for disinfection.

EXAMPLE B

9. Formulations B1 to B3 were prepared and tested on the gram positive surrogate *Staphylococcus aureus* (ATCC 6538) according to the ASTM Method E-2111-00. The results were compiled and are shown in Table B below.

TABLE B

	FORMULATION		
	B1	B2	B3
INGREDIENT	% w/w	% w/w	% w/w
Briquest ADPA-60AW (60% HEDP)	0.29	---	---
Briquest 301-50A (50% ATMP)	---	0.29	---
STPP (90% sodium tripolyphosphate)	---	---	0.29
C6 Dowfax Hydrotrope (45%)	0.08	0.08	0.08
Alfonic L610-3.5 (100% AE)	0.05	0.05	0.05
Hydrogen Peroxide (50%)	0.55	0.55	0.55
Biosoft S-100 (98% DDBSA)	0.18	0.18	0.18
pH	about 2	about 2	about 2
Water	to 100	to 100	to 100
Germicidal results			
ASTM Method E-2111-00 carrier test, Contact time 3 min.	6.85	6.85	6.56

Table B. Efficacy of formulations based on different phosphorous containing acids. Numbers shown under germicidal results are Log_{10} reduction in the number of viable organisms. A minimum of 6- Log_{10} reduction is considered effective as a disinfectant.

10. Formulations B1 to B3 are exemplary embodiments of the present invention containing 0.55 wt./wt. % hydrogen peroxide. These solutions result in a greater than 6 Log_{10} reduction in bacteria counts, showing them to be effective disinfectants.

11. Formulations B1, B2 and B3 differ only in the type of phosphorus based acid that is utilized. In formulation B1, the phosphorus-based acid employed is 1-hydroxyethylidene-1, 1,-diphosphonic acid (HEDP, a diphosphonic acid).

Formulation B2 has been prepared with amino tri(methylene phosphonic acid) (ATMP, a triphosphonic acid), while formulation B3 is not based on a phosphonic acid, but rather on another phosphorus-based acid, tripolyphosphoric acid (a polymerized form of phosphoric acid). These experiments demonstrate that other phosphorus-based acids, alone, can be employed in preparation of the claimed composition without a significant drop in biocidal efficacy.

EXAMPLE C

12. Formulations C1 to C9, based on 0.55 wt./wt.% hydrogen peroxide, were prepared and tested in accordance with ASTM Method E-2111-00 against *Staphylococcus aureus* (ATCC 6538). The only difference among formulations C1 to C9 is in the anionic surfactant used. Formulation C10 was prepared and tested as a control. The results are shown in Table C below.

TABLE C

	FORMULATION				
	C1	C2	C3	C4	C5
INGREDIENT – [%] w/w	% w/w	% w/w	% w/w	% w/w	% w/w
Phosphoric acid (75%)	0.11	0.11	0.11	0.11	0.11
Briquest ADPA-60AW (60%)	0.29	0.29	0.29	0.29	0.29
C6 Dowfax Hydrotrope (45%)	0.08	0.08	0.08	0.08	0.08
Alfonic L610-3.5 (100%)	0.05	0.05	0.05	0.05	0.05
Hydrogen Peroxide (50%)	0.55	0.55	0.55	0.55	0.55
Dowfax C10L C10 (45%)	---	0.18	---	---	---
Stepan Mild SL3 (SLSS) (32%)	---	---	0.17	---	---
Petro ULF (ANS) (95%)	---	---	---	0.17	---
Stepanol WAC (SLS) (29%)	---	---	---	---	0.16
Biosoft S-100 (98%)	0.18	---	---	---	---
pH	about 2	about 2	about 2	about 2	about 2
Water	to 100	to 100	to 100	to 100	to 100
Germicidal Results					
ASTM Method E-2111-00, Contact time 3 min; Staph. aureus	6.76	6.76	5.91	6.76	6.83

TABLE C – continued

	FORMULATION				
	C6	C7	C8	C9	C10
INGREDIENT – [%] w/w	% w/w	% w/w	% w/w	% w/w	% w/w
Phosphoric acid (75%)	0.11	0.11	0.11	0.11	0.11
Briquest ADPA-60AW (60% HEDP)	0.29	0.29	0.29	0.29	0.29
C6 Dowfax Hydrotrope (45%)	0.08	0.08	0.08	0.08	0.08
Alfonic L610-3.5 (100% AE)	0.05	0.05	0.05	0.05	0.05
Hydrogen Peroxide (50%)	0.55	0.55	0.55	0.55	0.55
Hostapur SAS-30 (30% SAS)	---	---	0.18	---	---
Standapol LF (35% SOS)	---	---	---	0.18	---
Alpha-Step MC-48 (37% SMSE/SFA)	---	0.16	---	---	---
Bioterge PAS-8 (SOS) (38%)	0.17	---	---	---	---
pH	about 2	about 2	about 2	about 2	about 2
Water	to 100	to 100	to 100	to 100	to 100
Germicidal Results					
ASTM Method E-2111-00, Contact time 3 min; Staph. aureus	6.76	6.21	5.45	6.4	NM

Table C. Efficacy of formulations based on different anionic surfactants. Numbers shown under germicidal results are Log_{10} reduction in the number of viable organisms. A minimum of 6-Log_{10} reduction is considered effective as a disinfectant. NM means not measurable due to substantial growth - plates had colonies too numerous to count (typically a reduction of less than 3logs).

13. All formulations C1 to C10 contain C6 alkylated sulfonated diphenyl oxide disodium salt which is both a hydrotrope and an anionic surfactant. This ingredient contributes to virucidal activity of the present inventive formulation, as will be discussed further below.

14. Formulation C1 contains dodecyl benzene sulfonic acid which belongs to the group consisting of C8 to C16 alkyl aryl sulfonic acids and alkali metal and ammonium salts thereof.

15. Formulation C2 contains C10 alkylated sulfonated diphenyl oxide disodium salt which belongs to the group defined as C8 to C22 alkyl diphenyl oxide sulfonic acids and alkali metal and ammonium salts thereof.

16. Formulation C3 contains disodium laureth sulfosuccinate and is based on an ethoxylated ester of sulfosuccinic acid, with a hydrophobic chain of equivalent length C8.

17. Formulation C4 contains sodium alkyl naphthalene sulfonate which belongs to the group defined as naphthalene sulfonic acids and alkali metal and ammonium salts thereof.

18. Formulations C5 and C9 contain sodium lauryl sulfate and sodium octyl sulfate, respectively, which belong to the class identified herein as alkali metal C8 to C18 alkyl sulfates. More specifically, formulation C5 is based on a sulfated C12 alcohol.

19. Formulations C6 and C8 contain sodium octyl sulfonate and sodium C14 - C17 sec-alkyl sulfonate, respectively, which belong to the group consisting of C8 to C22 alkyl sulfonic acids and alkali metal and ammonium salts thereof.

20. Formulation C7 contains a mixture of sodium methyl 2-sulfo C12-C16 ester and disodium 2-sulfo C12-C18 fatty acid salt which are members of the class consisting of sulfonated C12 to C22 carboxylic acids and alkali metal and ammonium salts thereof. More specifically, formulation C7 is an example of a sulfonated carboxylic acid and a sulfonated methyl-ester of that acid.

21. Formulations C1, C2, C4 and C6 comprise surfactants which have a sulfonic acid moiety as the polar head, and hydrophobic chains of lengths equivalent to C15-C16, C10-C12, C8-C12, and C8, respectively.

22. Formulations C1, C2, C4, C5, C6, C7 and C9 are all effective as disinfectants as they were able to achieve a greater than 6 $\log_{10}$ reduction in bacterial counts. Although formulations C3 and C8 are not so effective as to be considered disinfectants, they are nonetheless highly effective, achieving a greater than 5 $\log_{10}$ reduction in bacterial counts. In contrast, formulation C10 resulted in bacterial counts which were too numerous to count, i.e. less than a 3 $\log_{10}$ reduction in bacterial counts. The above tests show that formulations according to the present invention exhibit bactericidal activity.

EXAMPLE D

23. Formulations D1 to D3 according to the present invention were prepared and tested using ASTM Method E-2111-00 on the gram positive surrogate *Staphylococcus aureus* (ATCC 6538). The only difference among these formulations is the pH value and amount of NaOH buffer added to achieve the pH value. The results were compiled and are shown in Table D below.

TABLE D

	FORMULATION		
	D1	**D2**	**D3**
INGREDIENT	**% w/w**	**% w/w**	**% w/w**
Phosphoric acid (75%)	0.11	0.11	0.11
Briquest ADPA-60AW (60%)	0.29	0.29	0.29
C6 Dowfax Hydrotrope (45%)	0.08	0.08	0.08
Alfonic L610-3.5 (100%)	0.05	0.05	0.05
Hydrogen Peroxide (50%)	0.55	0.55	0.55
Biosoft S-100 (DDBSA) (98%)	0.18	0.18	0.18
NaOH (50%) to pH shown	pH 3.8	pH 5.0	pH 6.0
Water	to 100	to 100	to 100
Germicidal results			
ASTM Method E-2111-00 Contact time 3 min.	6.57	6.14	5.03

Table D. Efficacy of formulations at differing pH. Numbers shown under germicidal results are Log_{10} reduction in the number of viable organisms. A minimum of 6- Log10 reduction is considered effective as a disinfectant.

24. The results in Table D show that the composition is effective over a wide pH range up to at least 6.0. However, germicidal activity is seen to exhibit an inverse dependence on pH.

EXAMPLE E

25. Formulations E1 to E5 containing 0.55 wt./wt. % hydrogen peroxide, were tested against the non-enveloped polio virus ATCC VR-192 in accordance with the quantitative carrier test incorporating the essential requirements of the Canadian General Standards Boards' standard entitled Assessment of Efficacy of

Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices" (CGSB 1997). Stainless steel disks were used as the carrier surface for virucidal tests. Silk suture loops were not used because of the extreme difficulty in using them for standardized tests. Each stainless steel disk received test virus in bovine serum. After the inoculum had dried, it was exposed either to Earle's buffer solution or the test disinfectant for the required contact time and temperature. Each disk was placed in a vial with eluent/diluent and vortexed to recover the inoculum. The control and test eluates were inoculated into cell cultures for virus plaque assays. The plaque forming units (PFU) were then determined. To avoid false positive results, further controls were carried out by exposing the cell monolayers to a non-virucidal and non-cytoxic dilution of the test products and then using the same monolayers for plaque assays. If the number of plaques on such pre-exposed monolayers was the same as those exposed to Earle's solution, the product was regarded as free from interference. In the tests, there were three control carriers to every five test carriers. The results are included in Table E below.

TABLE E

	FORMULATION				
	E1	E2	E3	E4	E5
INGREDIENT – [%] w/w	% w/w	% w/w	% w/w	% w/w	% w/w
Phosphoric acid (75%)	0.11	0.11	0.11	0.11	---
Briquest ADPA-60AW (60%)	---	0.29	0.29	0.29	0.29
C6 Dowfax Hydrotrope (45%)	0.08	---	0.08	0.08	0.08
Alfonic L610-3.5 (100%)	0.05	0.05	---	0.05	0.05
Hydrogen Peroxide (50%)	0.55	0.55	0.55	0.55	0.55
Biosoft S-100 (DDBSA) (98%)	0.18	0.18	0.18	---	0.18
Phosphoric acid (75%) (for adjusting pH to about 1.8)	0.06	---	---	0.11	---
Water	to 100	to 100	to 100	to 100	to 100
Germicidal Results					
Quantitative virus test (CGSB 1997) Contact time 5 min. Log_{10} reduction in Polio Virus	1.72	0.08	4.12	4.55	4.24

Table E. Efficacy results versus polio virus. A minimum 3-Log_{10} reduction is considered effective as a virucide.

26. Only formulations E3 to E5 are effective as virucides since only they were able to achieve a greater than 3 $\log_{10}$ reduction in viral counts. Table E demonstrates that virucidal activity is highly dependant on the 1-hydroxyethylidene-1,1,-diphosphonic acid and the C6 alkylated sulfonated diphenyl oxide disodium salt, as shown by the results for formulations El and E2. Furthermore, formulation E4 shows that dodecylbenzene sulfonic acid is not required for virucidal activity. Formulation E3 shows that virucidal activity is not affected by the presence of the non-ionic surfactant, Alfonic L610-3.5 (C6 - C10 alkyl, 3.5 moles of ethylene oxide (EO) alcohol ethoxylate (AE)). Formulation E5 shows that phosphoric acid is not a requirement for virucidal activity.

27. I have reviewed U.S. application serial number 10/067,809 filed on 02/08/2002, the examiner's report dated 12/31/2002, the prior art cited in the report and in the Information Disclosure Statements filed to date, and the response to the examiner's report being filed concurrently herewith. I agree with the arguments and statements made in response.

28. This affidavit is made for the purpose of responding to the office action dated 12/31/2002 and for no improper purpose.

Sworn before me in the City of Toronto,)
Province of Ontario,) SS:
this 31st day of March, 2003.)

Jose A. RAMIREZ